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RESEARCH ARTICLE

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Enzyme-mediated biofilm inhibition of *Hedychium venustum* against *Pseudomonas aeruginosa*

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ABSTRACT: Bacteria produces biofilm to protect itself from adverse conditions. These biofilms are made of exopolysaccharides which safeguards it from host immune system and make it resistant to antibiotics. This causes most of the infections in human body. The autoinducers help to maintain the bacterial community at a threshold, this density dependent process is called quorum sensing. In this study, biofilm quantification was performed by crystal violet assay. At 40 mg/mL concentration, the *Hedychium venustum* hydro-methanolic crude extract (6:4) inhibited 40% of *Pseudomonas aeruginosa* biofilm. In fluorescence microscopic analysis, the dead cells in red colour revealed that the cells in the biofilm were affected by the plant extract. The scanning electron micrographs displayed that the coupon surface of untreated sample was rough since there were irregularities due to bacterial metabolism, while the coupon surface of treated sample was smooth due to the prevention of attachment of bacteria to the surface. Significant amylolytic activity with 62.183 U total enzymatic activity have correlation with the biofilm inhibition property. This is the first report on amylolytic and anti-biofilm activity of rhizome extract of *H. venustum* against *P. aeruginosa*. Since biofilms contribute significantly to the development of antimicrobial resistance, there is a necessity of alternative antimicrobial compounds that can inhibit biofilm formation. After further studies, the extract can also be considered a natural anti-biofilm agent.

KEYWORDS: Bacteria, hydro-methanolic extract, anti-biofilm activity, amylolytic property, antimicrobial agent.

INTRODUCTION

Bacterial biofilms are multi-microbial communities with three dimensional structure enclosed in a self-synthesized extracellular polymeric matrix [1, 2, 3]. They safeguard the bacteria from external environment, thereby making it resistant to the attack of the host immune system and antimicrobials [4, 5, 6]. According to Nassar et al. [7] 60-70% of hospital infections and 80% of human infections are due to bacterial biofilms. The endurance of the biofilm for a longer period could gradually promote the failure of the treatments provided for bacterial and other microbial infections. This leads to antibiotic resistance which is a major threat to living organisms. Thus, anti-biofilm agent which can prevent or inhibit biofilm formation was thought as an alternative to antimicrobial agent [1, 7, 8]. Therefore, effective and safe agents against microbes are much required to attack the multidrug resistant microorganisms [1, 9]. In this regard, different plants and herbs possessing biologically active compounds which had antimicrobial and anti-biofilm properties with less side effects (negative) on the health of human beings are considered [1, 9, 10]. In order to reduce or inhibit biofilm formation, studies were conducted on quorum sensing (QS) and quorum quenching [11].

Quorum sensing (QS) is a density dependent mechanism, where the signalling molecules called autoinducers keep the bacterial community under a threshold level [11, 12]. Meanwhile, quorum quenching is the inhibition of quorum sensing, which disables the bacteria's ability to generate virulent expressions and it does not affect the bacterial growth. This consequently exposes the bacteria to the defence mechanism of the host for an adequate period of time which helps to eradicate the bacteria [11, 13]. Therefore, inhibition of quorum sensing is much need and its approaches are classified into enzymatic and non-enzymatic inhibition. The non-enzymatic approaches involve (i) inhibition of signal molecule synthesis, (ii) blocking the reception of signal by modification and conformation changes in the signal molecule that binds to the response gene and (iii) by allosteric or competitive inhibition of signal molecule that binds to the response gene. By these ways bacterial silencing can be attained [14, 15]. In enzymatic inhibition, the

autoinducers are degraded which gradually deactivates the signalling cascade. Examples of some enzymes which degrades the signalling molecules are acylase, lactonase, oxidoreductase, etc. [15, 16]. The extracellular polymeric matrix present in the biofilm are made of carbohydrates, mainly polysaccharides. Starch is a complex carbohydrate or a polysaccharide made of glucose monomers attached by glycosidic bonds [17].

The amylase enzyme converts the starch into glucose by acting on the glycosidic bond of the substrate. Since the biofilms are made of exopolysaccharides, the biofilms are degraded by amylolytic activity [18, 19]. In the present investigation, the rhizome extract of *Hedychium venustum* Wight, was studied for its amylolytic mediated biofilm inhibition. *Hedychium*, a genus familiar as ginger lilies, with thick, branched and fleshy rhizome, belongs to Zingiberaceae family [20]. The members of Zingiberaceae are well known for its medicinal properties and it is mostly found in tropics, especially in Southeast Asia. It is the main source for medicinal products, food, perfume, dyes, spices and aesthetics [21, 22]. *H. venustum* is a rhizomatous endemic plant studied for its pharmacological and phytochemical properties [20, 23, 24]. However, the amylolytic activity and anti-biofilm activity of *H. venustum* against *Pseudomonas aeruginosa* has never been explored. Therefore, the possibility of it being used as an alternative to antibiotics for reducing the biofilm-associated infections can be explored.

MATERIALS AND METHODS

Plant material - collection and identification

During January 2021, the rhizomes of *H. venustum* were collected from Idukki district, Kerala, India. These plants collected were identified and authenticated by Dr. Bince Mani, St. Thomas College, Palai, Kerala and the voucher specimen number RHT67230 was assigned to the plant; its herbarium is maintained at Rapinat Herbarium, Trichy. The rhizomes were uprooted carefully without causing any damage. The soil particles adhered to the rhizome were cleaned and the rhizomes were kept in plastic bags and transferred to laboratory. Then the rhizomes were removed from the bags and were stored at room temperature.

Extraction process

The rhizomes of the plant material were washed properly and sliced into small pieces. These were shade dried and ground to coarse powder using a mixer. The powder was stored in an air tight container. For the extraction process, dried powder (40g) was soaked in 450 ml of methanol and water in 6:4 by shaking on a mechanical shaker and kept in refrigerator overnight. After 2 days, the plant extract was filtered with Whatman filter paper. The resulting filtrate was concentrated at 50º C to obtain crude hydro-methanolic extract.

Analysis of biofilm inhibition by extract

The extract was tested for presence of anti-biofilm activity using prevalent bacterial strain *Pseudomonas aeruginosa* strain jp07 (MF426269) purchased from Bioresource Technology Lab, Department of Biotechnology, Thiruvalluvar University, India. Sodium acetate, nutrient broth, 1X PBS, Crystal violet solution and Gentamicin antibiotic solution were purchased from Hi-media, India. 96 sterile well plates were from Tarsan, India and conical flask were purchased from Borosil.

Microtitre plate assay (MTP) for Biofilm inhibition

Through microtitre plate assay the efficiency of the rhizome extract to inhibit the biofilm was studied. This assay was performed by slightly modifying the method described by Vadakkan et al. [15]. 96 welled-flat bottom polysterene titre plates were used for this assay as these plates function as a surface for adhesion of bacteria. 180 µL BHI broth was added to each well, then subsequently 10 µL overnight culture of *P. aeruginosa* was inoculated to it. 10 µL of extract from the stock solution of 40 mg/mL concentration was added to the above mentioned broth and culture mixture in each well. The well containing the BHI broth and culture but lacking the extract is maintained as Control. The whole setup of the assay was incubated for 24 h at 37ºC. After 24 h the wells were emptied by removing the contents. Then it was washed using 0.2 mL of PBS (Phosphate buffer saline) with pH 7.2 in order to remove the free floating and weakly adhered bacteria in the well. The bacterial adherence was stabilized with 2% sodium acetate. 0.1%, w/v of crystal violet was used for staining. Excessive stain was removed using deionized water and the plates were dried. Finally, using 95% ethanol these dried plates were washed. Then by using microtitre plate reader (Thermo) the optical density was measured at 600 nm. The biofilm inhibition percentage was the calculated from the equation given below and from the graph.

$$\% \text{ Biofilm inhibition} = \frac{\text{Control OD} - \text{Test OD}}{\text{Control OD}} \times 100$$

Biofilm inhibition analysis by EtBr/AO staining

The EtBr/AO staining was detected using Fluorescence microscopic analysis of biofilm inhibition. About 5 x 10^6

cells/ml of *P. aeruginosa* were plated in a 24 well plate on a coverslip and it was treated with 87.76 µg/ml of plant extract (sample) in a nutrient broth. The plate was incubated for 48 hours in an incubator at 37°C. 50 µl of 1 mg/ml acridine orange and ethidium bromide were added to the wells after incubation and mixed gently. Then finally, the plate was centrifuged for 2 minutes at 800 rpm and assessed immediately within an hour. At least 100 cells were studied by a fluorescence microscope (Labomed TCM 400) using a fluorescent filter [25].

Surface morphology analysis of biofilm inhibition by electron microscopy

By modifying the method used by Dey et al., 2020, determination of surface morphology of biofilm inhibition was performed by stainless steel coupon as a surface. In a nutrient broth medium, the fresh culture of *P. aeruginosa* cells was incubated together with sterile stainless steel coupon. For the treated sample 5 ml of 40 mg/mL concentration of plant extract (1:19 dilution) was added to it. The untreated sample was prepared the same way except for adding the extract. When the completion of incubation at 37°C for 28 days in a shaking incubator at 150 RPM, scanning electron microscopy were utilized for the surface morphology analysis of biofilm inhibition of the samples [26].

Analysis of amylolytic activity

Starch was used as a substrate for the spectrophotometric analysis of amylolytic property of rhizome extract of *H.venustum*. 0.2, 0.4, 0.6, 0.8 and 1 ml volume of 40 mg/mL concentration of plant extract diluted to 1:19 ratio was added to 1 ml of 1% starch and all solutions were made up to 2 ml using distilled water and incubated for 30 minutes at 37 ºC. In control setup instead of plant extract, distilled water was added. After incubation, 1ml of DNS reagent was administered to the solution to stop the reaction. Then the solution was kept for 20 minutes in hot water bath and to all solutions 7.5 ml distilled water was added. Using UV-Vis spectrophotometer (ELICO-SL218), the absorbance at 540 nm was measured for each test sample of different concentration [27]. A standard curve constructed with maltose is utilized to determine the amylolytic activity. The amount of enzyme that liberates 1µmol of glucose equivalent per minute under the assay conditions was defined as one unit of amylase activity (U/ml). The specific activity was derived from the acquired value by dividing the total activity by total protein content [28].

Statistical analysis

Statistical analysis of the data collected from the study was conducted by one-way ANOVA (Analysis of Variance) followed by Dunnett's post-test using Graph-pad prism Version 5.01 software.

RESULTS

Spectrophotometric analysis of biofilm inhibition

The bacterial biofilm inhibition by the plant extract was studied spectrophotometrically. According to the results, when the concentration of the extract increased, the biofilm production was significantly reduced (Figure 1). At higher concentration of 40 mg/mL, maximum biofilm inhibition of 40% was observed. Untreated sample had 100% biofilm formation and in treated sample the biofilm formation was reduced by 40% from control. The result proved that the concentration of the plant extract is directly proportional to the biofilm inhibition.

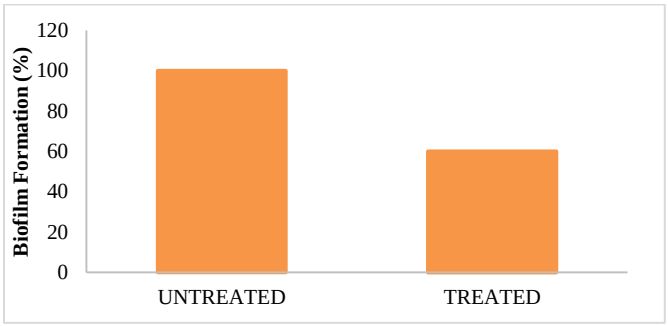
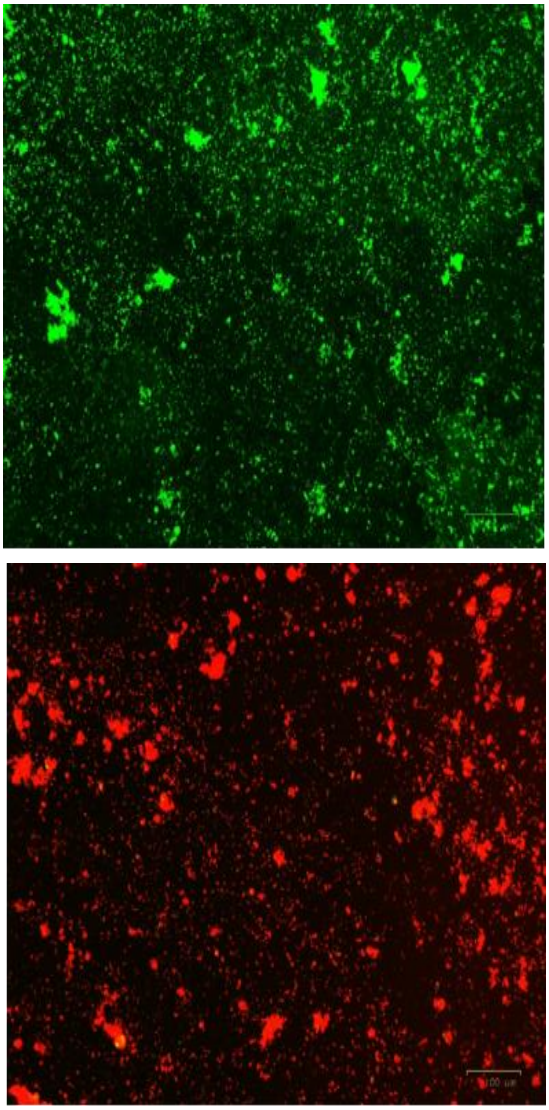


Figure 1. Biofilm inhibition by extract.

Fluorescence microscopic analysis of biofilm inhibition

EtBr/AO staining detected the surface topology of biofilm inhibition caused by the plant extract. This was visualized using fluorescence microscope (Figure 2). Ethidium bromide entered only the dead cells and produced red fluorescence whereas acridine orange penetrated the live cells with cell membrane and it emitted green fluorescence [29]. The test sample treated with the plant extract showed red coloured cells in loosely packed appearance while the untreated control sample displayed less loosely arranged green coloured cells. The presence of red coloured cells in the treated sample denoted the dead cells which was due to biofilm damage. This data proved that the presence of extract caused less cell adhesion on the surface which lead to the reduction of the biofilm.



Untreated

Treated

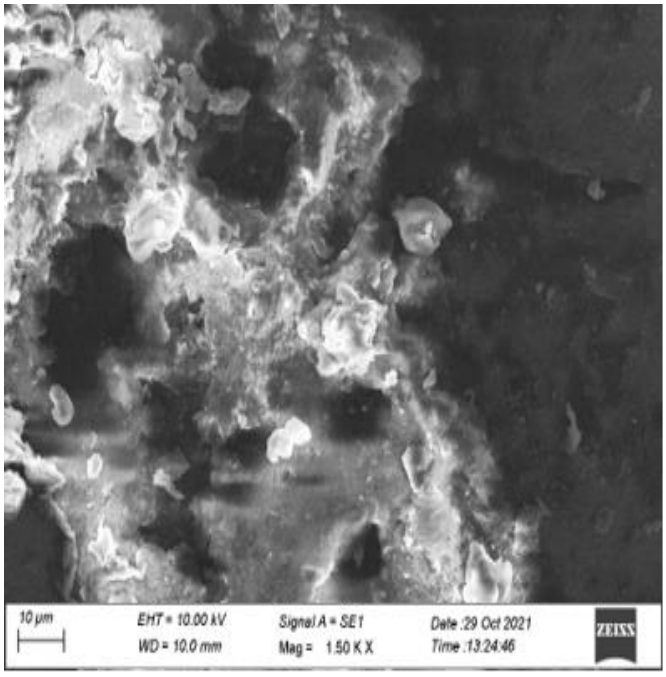
Figure 2. Fluorescence microscopic analysis of biofilm inhibition.

Surface morphology analysis of biofilm inhibition

The surface morphology of bacterial biofilm inhibition was analysed from the scanning electron micrography (Figure 3). The micrograph of the untreated sample revealed deep grooves and infoldings on the surface of the stainless steel coupon which was because of the degradation caused by the bacterial biofilm. In contrary to this, the treated sample micrograph displayed plane, flat and smooth surface since there was absence of degradation caused by biofilm. Hence it was apparent that during the absence of the plant extract bacteria was able to attach to the surface whereas the addition of plant extract inhibited the attachment of bacteria to the surface.

Amylolytic activity

The hydro-methanolic rhizome extract of *H. venustum* showed significant amylolytic activity with 62.183 U total enzymatic activity and 628.113 U/mg specific enzyme activity at extract concentration of 40 mg/mL diluted to 1:19 ratio. The enzyme activity was found to be correlated to the extract concentration. The amylolytic action of the rhizome extract converts the starch (substrate) into sugars. Similarly, it could be considered as the reason for the conversion of the polysaccharides present in the biofilm into sugars. This might cause damage to the biofilm and it could make the bacterial cells susceptible.



Untreated

Treated

Figure 3. Surface morphology analysis of biofilm inhibition.

DISCUSSION

In the present investigation the hydro-methanolic (6:4) crude extract of *H. venustum* rhizome produced maximum biofilm inhibition of 40% at 40 mg/mL concentration against *P. aeruginosa*. In comparison, the plant rhizome extract of *H. forrestii var. forrestii* demonstrated about 80% biofilm inhibition against *Staphylococcus aureus* at a concentration of 500 µg/ml [30]. Linalool from the essential oil of rhizome of *H. larsenii* at 0.004% concentration produced maximum of 91% biofilm inhibition against *Streptococcus pyogenes* [31]. Similarly, at the concentration of 100 µg/ml, curcumin a major constituent of *Curcuma longa* efficiently inhibited the biofilm formation by 52%, 89%, 52% and 76% in uropathogens, such as *Escherichia coli*, *Pseudomonas aeruginosa* PAO1, *Proteus mirabilis* and *Serratia marcescens* respectively [32]. A study on *Zingiber officinale* rhizome extract exhibited 92.96% biofilm inhibition against *Helicobacter pylori* at 50 µg/mL concentration [33]. Another study on *Z. officinale* root extract at 1-10% concentration reduced biofilm development of *Pseudomonas aeruginosa* PA14 by 39-56% [34]. Compared to the biofilm inhibition percentage of other plant extracts and essential oils, *H. venustum* also exhibited significant biofilm inhibition.

According to the fluorescence microscopic analysis, in the treated sample dead cells were observed in a loosely packed appearance. It is considered as a result of biofilm damage. This is due to the action of anti-biofilm components present in the extract which acts against the biofilm and biofilm forming bacteria. Thus, biofilm damage makes the bacteria susceptible to these bioactive components, since the biofilm served as a protective shield which provided antibiotic resistance. In accordance to the present study, a previous study on the plant rhizome extract of *H. forrestii var. forrestii* against *S. aureus* reported the observation of dead cells in the treated sample [30]. Correspondingly, an exposure of curcumin from *C. longa* against *S. aureus* at different concentration such as 25 µM, 50 µM and 100 µM resulted in the observation of 63%, 75% and 90% dead cells, respectively [35]. In a previous study, the effect of curcumin on *Porphyromonas gingivalis* biofilm was investigated. In the results observed, the control sample appeared green denoting live cells and the treated sample appeared red denoting dead cells [29]. The fluorescence microscopic observation in this study also points out similar results from the previous studies, where the presence of plant extract causes the death of bacterial cells and less cell adherence to the surface.

In the present study, the scanning electron micrograph revealed that the surface of the coupon in the treated sample was smooth while in the untreated sample was rough. This was due to the inability of the bacteria to attach to the surface, when treated with the extract. A similar observation was seen in the ginger extract which inhibited the biofilm of *P. aeruginosa* by detaching the biofilm cells from the surface [34]. The roughness and irregularities on the coupon surface in the untreated sample was caused due to the bacterial metabolism [36]. *Comparably, the exposure of* the rhizome extract of *H. forrestti var. forrestii* against *S. aureus* exhibited smooth coupon surface without any bacterial degradation since the bacteria turned out to be incompetent to attach over the surface. The untreated sample showed grooves and invaginations on the surface due to the presence of biofilm and bacterial degradation [30]. In the same way, a study conducted on different herbaceous plant extracts, the treatment of turmeric extract and green tea extract against sulphate reducing bacteria was reported to show even and less clumsy coupon surface while the surface of the untreated coupon was uneven which indicated highly corroded surface [37]. The scanning electron micrograph of the present study is in accordance with the result of fluorescence microscopic observation, supporting the fact that the incompetence of the bacterial cells to attach to the surface is caused due to the presence of plant extract.

Biofilms are made of polysaccharides which serves as a substrate for amylolytic enzymes which acts on the substrate's glycosidic bond [18, 19]. The enzyme amylase breakdown or convert starch into glucose [17]. This causes damage to the polysaccharides in the biofilm. The bacteria will become susceptible to the antimicrobial agents and antibiotics when the biofilm is degraded. Biofilm maintains the bacterial population at a threshold level. This process improves the quorum sensing and therefore has prominent role in its virulence [38]. The plant extract affects the threshold level of the bacterial population. Therefore, this study supported the above theory, revealing that the rhizome extract of *H. venustum* exhibited amylase dependent biofilm inhibition against *P. aeruginosa* with 62.183 U total enzymatic activity. Similarly, the plant rhizome extract of *H. forrestti var. forrestii* showed amylolytic dependent biofilm inhibition against *S. aureus* with 110.916 U total enzyme activity [30]. The findings of a group of researchers, suggested the possibility of *Elletaria cardamomum* essential oil to be used as a safe antimicrobial agent against the biofilms formed by Gram-negative pathogens like *E. coli* and *P. aeruginosa* [1]. The anti-biofilm property of the extract provides the scientific justification as a hopeful anti-biofilm treatment. Further *in vivo* evaluation is needed for the potential application of the extract in treatment of biofilm associated infections. The purified compounds will have

more potential to inhibit biofilm than the crude extract. Therefore, more studies are required to identify, purify and isolate the bioactive compound from crude extract to know the exact biological mechanism.

CONCLUSIONS

The study displayed the anti-biofilm and amylolytic activity of rhizome extract of H. venustum against *P. aeruginosa* biofilm. The spectrophotometric data revealed that compared to untreated sample, the treated sample showed 40% reduction in biofilm formation i.e. 40% of biofilm was inhibited. The fluorescence microscopic analysis aided to identify the effect of biofilm inhibition on the bacterial cell adherence. The micrograph disclosed the biofilm architecture of the stainless steel coupon, in which the plant extract exhibited incredible activity by preventing the corrosion or degradation of the coupon; whereas in untreated sample the coupon was corroded by the biofilm. The amylolytic activity of the plant suggested that, the biofilm inhibition was caused by amylolytic mediated activity. Further investigations on this plant extract would help us to reveal the bioactive compound responsible for the amylolytic and anti-biofilm activity; and to understand the exact mechanism occurring during the activity. This would help to find a possible replacement to antibiotics with a natural bioactive plant derived compound having anti-biofilm activity with reduced infection recurrence and side effects.

DECLARATION

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Authorship contributions

Concept: K.V., Design: K.V., Data Collection or Processing: S.V., Analysis or Interpretation: S.V., K.V., Literature Search: S.V., M.K.C., Writing: S.V., B.M., M.K.C.

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Competing interests

The authors declared that there is no conflict of interest.

REFERENCES

[1] Abdullah, Asghar, A., Algburi, A., Huang, Q., Ahmad, T., Zhong, H., Javed, H. U., Ermakov, A. M., Chikindas, M. L. (2021): Anti-biofilm potential of *Elletaria cardamomum* essential oil against *Escherichia coli* O157:H7 and *Salmonella Typhimurium* JSG 1748. Frontiers in Microbiology 12: 620227. https:/doi.org/10.3389/fmicb.2021.620227

[2] Jamal, M., Ahmad, W., Andleeb, S., Jalil, F., Imran, M., Nawaz, M. A., Hussain, T., Ali, M., Rafiq, M., Kamil, M. A. (2018): Bacterial biofilm and associated infections. Journal of Chinese Medical Association 81(1): 7-11. https://doi.org/10.1016/j.jcma.2017.07.012

[3] Miquel, S., Lagrafeuille, R., Souweine, B., Forestier, C. (2016): Anti-biofilm activity as a health issue. Frontiers in Microbiology 7: 592. https://doi.org/10.3389/fmicb.2016.00592

[4] Rahman, M. R. T., Lou, Z., Yu, F., Wang, P., Wang, H. (2017): Anti-quorum sensing and anti-biofilm activity of *Amomum tsaoko* (Amommum tsao-ko crevost et lemarie) on foodborne pathogens. Saudi Journal of Biological Sciences 24(2): 324-330. https://doi.org/10.1016/j.sjbs.2015.09.034

[5] Solano, C., Echeverz, M., Lasa, I. (2014): Biofilm dispersion and quorum sensing. Current Opinion in Microbiology 18, 96-104. https://doi.org/10.1016/j.mib.2014.02.008

[6] Srey, S., Jahid, I. K., Ha, S.-D. (2013): Biofilm formation in food industries: a food safety concern. Food Control 31(2): 572-585. https://doi.org/10.1016/j.foodcont.2012.12.001

[7] Nassar, R., Hachim, M., Nassar, M., Kaklamanos, E. G., Jamal, M., Williams, D., Senok, A. (2021): Microbial metabolic genes crucial for *S. aureus* biofilms: an insight from re-analysis of publicly available microarray datasets. Frontiers in Microbiology 11: 607002. https://doi.org/10.3389/fmicb.2020.607002

[8] Livermore, D. M. (2011): Discovery research: the scientific challenge of finding new antibiotics. Journal of Antimicrobial Chemotherapy 66(9): 1941-1944. https://doi.org/10.1093/jac/dkr262

[9] Newman, D. J., Cragg, G. M. (2016): Natural products as sources of new drugs from 1981 to 2014. Journal of Natural Products 79(3): 629-661. https://doi.org/10.1021/acs.jnatprod.5b01055

[10] Abdullah, Algburi, A., Asghar, A., Huang, Q., Mustfa, W., Javed, H. U., Zehm, S., Chikindas, M. L. (2020): Black cardamom essential oil prevents *Escherichia coli* O157:H7 and *Salmonella Typhimurium* JSG 1748 biofilm formation through inhibition of quorum sensing. Journal of Food Science and Technology. 58: 3183-3191. https://doi.org/10.1007/s13197-020-04821-8

[11] Vadakkan, K., Vijayanand, S., Hemapriya, J., Gunasekaran, R. (2019a): Quorum sensing inimical activity of *Tribulus terrestris* against gram negative bacterial pathogens by signalling interference. 3 Biotech 9: 163. https://doi.org/10.1007/s13205-019-1695-7

[12] Waters, C. M., Bassler, B. L. (2005): Quorum sensing: cell-to-cell communication in bacteria. Annual Review of Cell and Developmental Biology 21: 319-346. https://doi.org/10.1146/annurev.cellbio.21.012704.131001

[13] Chen, F., Gao, Y., Chen, X., Yu, Z., Li, X. (2013): Quorum quenching enzymes and their application in degrading signal molecules to block quorum sensing-dependent infection. International Journal of Molecular Sciences 14(9): 17477-17500. https://doi.org/10.3390/ijms140917477

[14] Basavaraju, M., Sisnity, V. S., Palaparthy, R., Addanki, P. K. (2016): Quorum quenching: signal jamming in dental plaque biofilms. Journal of Dental Sciences 11(4): 349-352. https://doi.org/10.1016/j.jds.2016.02.002

[15] Vadakkan, K., Hemapriya, J., Selvaraj, V. (2019b): Quorum quenching intervened in vivo attenuation and immunological clearance enhancement by *Solanum torvum* root extract against

Pseudomonas aeruginosa instigated pneumonia in Sprague Dawley rats. Clinical Phytoscience 5: 24. https://doi.org/10.1186/s40816-019-0120-4

[16] Torres, M., Uroz, S., Salto, R., Fauchery, L., Quesada, E., Llamas, I. (2017): HqiA, a novel quorum-quenching enzyme which expands the AHL lactonase family. Scientific Reports 7: 943. https://doi.org/10.1038/s41598-017-01176-7

[17] Fatema, F., Khan, Z. H., Khan, N. D., Mular, S. M. (2017): Determination of amylase activity from germinated *Syzygium cumini* seed (jamun). International Journal of Applied Research 3(1): 573-575.

[18] Gopinath, S. C. B., Anbu, P., Arshad, M. K. M., Lakshmipriya, T., Voon, C. H., Hashim, U., Chinni, S. V. (2017): Biotechnological processes in microbial amylase production. BioMed Research International 2017: 1272193. https://doi.org/10.1155/2017/1272193

[19] Vani, S., Vadakkan, K., Mani, B. (2023): A narrative review on bacterial biofilm: its formation, clinical aspects and inhibition strategies. Future Journal of Pharmaceutical Sciences 9(50). https://doi.org/10.1186/s43094-023-00499-9

[20] Sabulal, B., George, V., Dan, M., Pradeep, N. S. (2007): Chemical composition and antimicrobial activities of the essential oils from the rhizomes of four *Hedychium* species from south india. Journal of Essential Oil Research 19(1): 93-97. https://doi.org/10.1080/10412905.2007.9699237

[21] Jantan, I. Bin, Yassin, M. S. M., Chin, C. B., Chen, L. L., Sim, N. L. (2003): Antifungal activity of the essential oils of nine Zingiberaceae species. Pharmaceutical Biology 41(5): 392-397. https://doi.org/10.1076/phbi.41.5.392.15941

[22] Tushar, Basak, S., Sarma, G. C., Rangan, L. (2010): Ethnomedical uses of Zingiberaceous plants of northeast india. Journal of Ethnopharmacology 132(1): 286-296. https://doi.org/10.1016/j.jep.2010.08.032

[23] Binimol, K. J., Vani, S., Thomas, S., Mani, B. (2022): Chemical principles, bioactivity, and pharmacology of two ginger lilies *Hedychium forrestii* Diels and *Hedychium venustum* Wight (Family: Zingiberaceae). In: Pullaiah, T. (ed.) Bioactives and Pharmacology of Medicinal Plants Vol. 2, Apple Academic Press, pp. 25-37. https://doi.org/10.1201/9781003281702-3

[24] Sabu, M. (2006): Zingiberaceae and Costaceae of south india. Indian Association for Angiosperm Taxonomy, Kerala.

[25] Bogachev, M. I., Volkov, V. Y., Markelov, O. A., Trizna, E. Y., Baydamshina, D. R., Melnikov, V., Murtazina, R. R., Zelenikhin, P. V, Sharafutdinov, I. S., Kayumov, A. R. (2018): Fast and simple tool for the quantification of biofilm-embedded cells sub-populations from fluorescent microscopic images. PLOS One 13(5): e0193267-e0193267. https://doi.org/10.1371/journal.pone.0193267

[26] Dey, A. S., Bose, H., Mohapatra, B., Sar, P. (2020): Biodegradation of unpretreated Low-Density Polyethylene (LDPE) by *Stenotrophomonas* sp. and *Achromobacter* sp., isolated from waste dumpsite and drilling fluid. Frontiers in Microbiology 11: 603210. https://doi.org/10.3389/fmicb.2020.603210

[27] Jain, A., Jain, R., Jain, S. (2020): Determination of Salivary Amylase Activity. In: Jain, A., Jain, R., Jain, S. (eds.) Basic techniques in biochemistry, microbiology and molecular biology: principles and techniques, Springer Protocols Handbooks. Humana, New York, NY: Springer US, pp. 223-225. https://doi.org/10.1007/978-1-4939-9861-6_52

[28] Vadakkan, K., Cheruvathur, M. K., Chulliparambil, A. S., Francis, F., Abimannue, A. P. (2021): Proteolytic enzyme arbitrated antagonization of helminthiasis by *Cinnamomum cappara* leaf extract in *Pheretima posthuma*. Clinical Phytoscience 7: 23. https://doi.org/10.1186/s40816-021-00261-9

[29] Kumbar, V. M., Peram, M. R., Kugaji, M. S., Shah, T., Patil, S. P., Muddapur, U. M., Bhat, K. G. (2021): Effect of curcumin on growth, biofilm formation and virulence factor gene expression of *Porphyromonas gingivalis*. Odontology 109(1): 18-28. https://doi.org/10.1007/s10266-020-00514-y

[30] Karayamparambil, B. J., Vadakkan, K., Thomas, S. (2024): Amylolytic-dependent biofilm inhibition of *H. forrestti var. forrestii* rhizome extract against *S.aureus* pathogens. Journal of Natural Pesticide Research 8: 100072. https://doi.org/10.1016/j.napere.2024.100072

[31] Praseetha, S., Sukumaran, S. T., Dan, M., Augustus, A. R., Pandian, S. K., Sugathan, S. (2023): The anti-biofilm potential of linalool, a major compound from *Hedychium larsenii*, against *Streptococcus pyogenes* and its toxicity assessment in *Danio rerio*. Antibiotics 12(3): 545. https://doi.org/10.3390/antibiotics12030545

[32] Packiavathy, I. A. S. V., Priya, S., Pandian, S. K., Ravi, A. V. (2014): Inhibition of biofilm development of uropathogens by curcumin - An anti-quorum sensing agent from *Curcuma longa*. Food Chemistry 148: 453-460. https://doi.org/10.1016/j.foodchem.2012.08.002

[33] Elbestawy, M. K. M., El-Sherbiny, G. M., Moghannem, S. A., Farghal, E. E. (2023): Antibacterial, antibiofilm, and anti-inflammatory activities of ginger extract against *Helicobacter pylori*. Microbiology Research 14(3): 1124-1138. https://doi.org/10.3390/microbiolres14030075

[34] Kim, H., Park, H. (2013): Ginger extract inhibits biofilm formation by *Pseudomonas aeruginosa* PA14. PLOS One 8(9): 1-16. https://doi.org/10.1371/journal.pone.0076106

[35] Tyagi, P., Singh, M., Kumari, H., Kumari, A., Mukhopadhyay, K. (2015): Bactericidal activity of curcumin I is associated with damaging of bacterial membrane. PLOS One 10(3): e0121313. https://doi.org/10.1371/journal.pone.0121313

[36] Feng, G., Cheng, Y., Wang, S.-Y., Borca-Tasciuc, D. A., Worobo, R. W., Moraru, C. I. (2015): Bacterial attachment and biofilm formation on surfaces are reduced by small-diameter nanoscale pores: how small is small enough?. NPJ Biofilms and Microbiomes 1: 15022. https://doi.org/10.1038/npjbiofilms.2015.22

[37] Md Zain, W. S., Hairul Salleh, N. I., Abdullah, A. (2018): Natural biocides for mitigation of sulphate reducing bacteria. International Journal of Corrosion 2018: 3567569. https://doi.org/10.1155/2018/3567569

[38] Vadakkan, K., Choudhury, A. A., Gunasekaran, R., Hemapriya, J., Vijayanand, S. (2018): Quorum sensing intervened bacterial signaling: pursuit of its cognizance and repression. Journal of Genetic Engineering and Biotechnology 16(2): 239-252. https://doi.org/10.1016/j.jgeb.2018.07.001